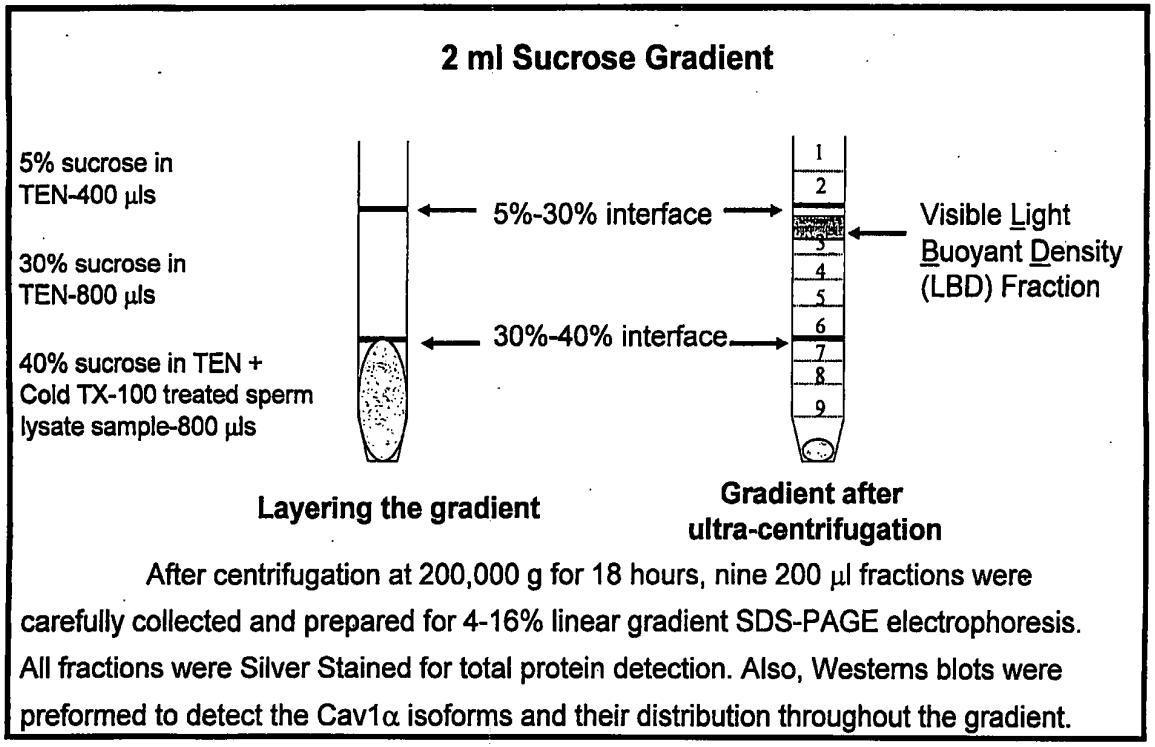
FIG. 1

PREPARATION, ULTACENTRIFUGATION AND COLLECTION OF THE SUCROSE GRADIENT : VISUAL IDENTIFICATION OF LIGHT BUOYANT DENSITY (LBD) FRACTIONS



After centrifugation at 200,000 g for 18 hours, nine 200 µl fractions were carefully collected and prepared for 4-16% linear gradient SDS-PAGE electrophoresis. All fractions were Silver Stained for total protein detection. Also, Westerns blots were preformed to detect the Cav1α isoforms and their distribution throughout the gradient.

FIG. 2

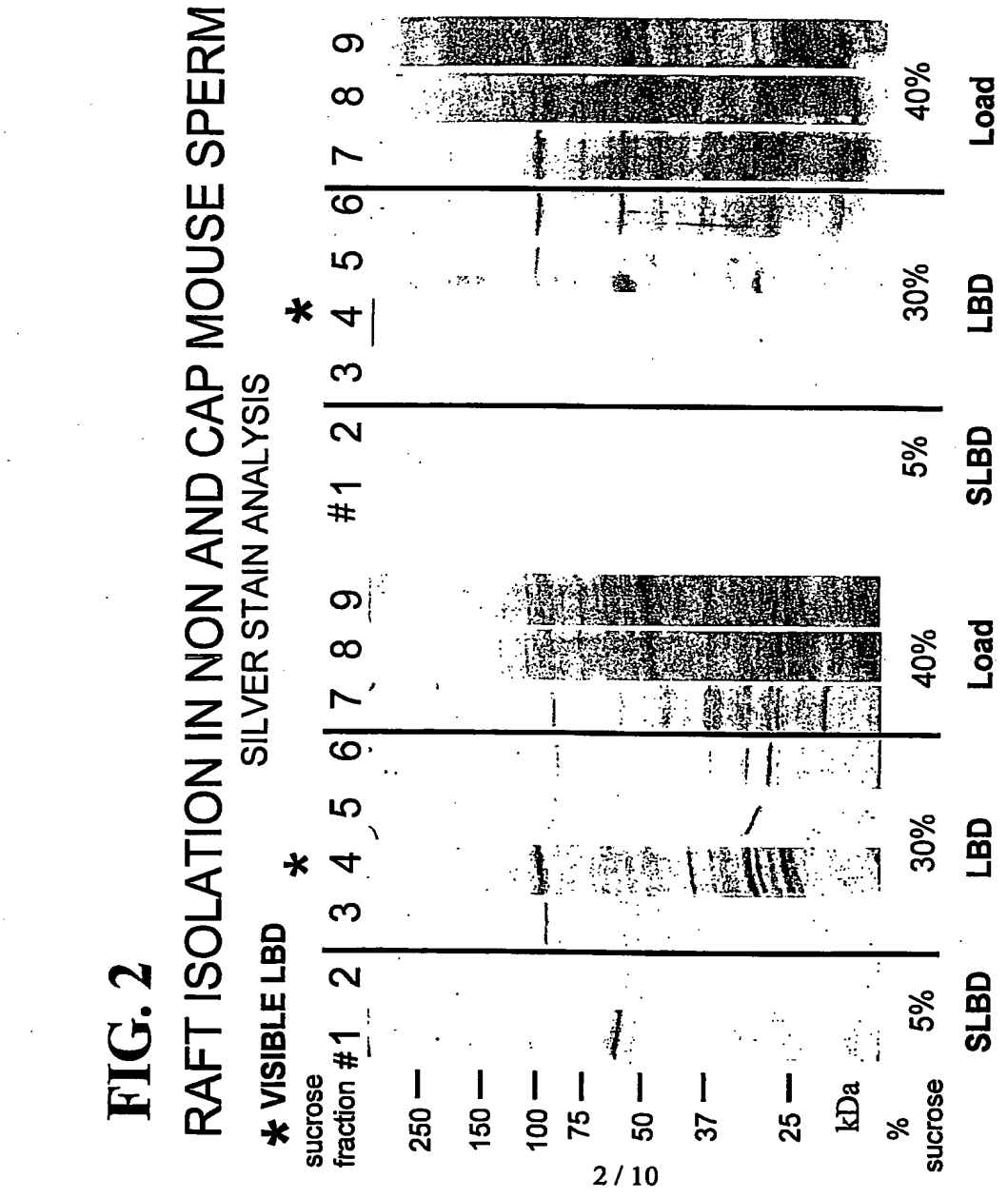

RAFT ISOLATION IN NON AND CAP MOUSE SPERM
SILVER STAIN ANALYSIS
* VISIBLE LBD
sucrose fraction #1 2 3 4 5 6 7 8 9 #1 2 3 4 5 6 7 8 9
250
150
100
75
50
37
25
kDa
% sucrose 5% 30% 40% 5% 30% 40%
SLBD LBD Load SLBD LBD Load
NONCAPACITATED
CAPACITATED

FIG. 3

INITIAL MASS SPEC IDENTIFICATION OF HYPOTHETICAL PROTEIN "BAND 5"

Band 5 (KD1-39-3)
>gi|12840105|dbj|BAB24761.1| (AK006830) evidence:NAS~hypothetical protein~putative [Mus musculus] [MASS=44885]

MGPHFTLLLA ALANCLCPGR PCIKCDQFVT DALKTFENTY LNDHLPHDIH KNVMRMVNHI
VSSFGVVTSA EDSYLGAVDE NTLEQATWSF LKDLKRITDS DLKGELFIKE LLWMLRHQKI
IFNNLARQFQ KEVLCPNKCG VMSQTLIWCL KCEKQLHICR KSLDCGERHI EVHRSEDLV
DCLLSWHRAS KGLTDYSFYR VWENSSETLI AKGKEPYLTK SMVGPEDAGN YRCVLDTIN
GHATVIRYDV TVLPPKHSEE NQPPNIITQE EHETPVHVTP QTPPGQEPES ELYPELHPE
YPELIPTVAQ NPEKKMKTRL LILLTLGFVV LVASIIISVL HFRKVSAKLK NASDEVKPT
SGSKSDQSLS QQMGLKKASQ ADFNSDYSGD KSEATEN

>monoisotopic mass = 44838

position	sequence (NCBI BLAST link)
25- 34	CDQFVTDALK
192- 200	GLTDYSFYR
221- 232	SMVGPEDAGNYRC
233- 247	CVLDTINQGHATVIR
351- 364	NASDEVKPTASGSK

BASED UPON EST/cDNA In the DATABASE

} ACTUAL PEPTIDES ID'ED BY MASS SPEC

SMVGPEDAGNYRC **BLASTP SUGGESTS PEPTIDE IS UNIQUE TO BAND**

FIG. 4

BIOINFORMATICS SUMMARY

PROTEIN and NUCLEOTIDE BLASTS
The only protein that matches with a significant E value to the mouse band 5 is a hypothetical human ortholog. Also true at the EST/cDNA level as demonstrated by the nucleotide BLASTN.

PROTEIN	aa	pMW	pI	TM	S-S	Domains	ID#
Mouse	397	44885	5.9	2	3	Ig-like	BAB24761.1
Human	350	38958	6.0	2	3	Ig-like	NP_872381

GENE	Chromosome	Exons	EST Source	Accession#
Mouse	7 B2	10	Testis cDNA, full-length insert RIKEN; 1479 bp	AK006830
Human	19q13.33	8	Adult brain medulla mRNA; 1695 bp	NM_182575

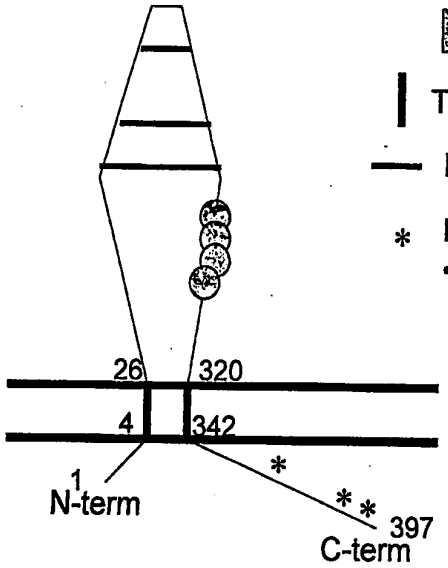
FIG. 5

PROTEIN MODEL BASED ON BIOINFORMATICS

PROTEIN LEVEL

1° •397 aa-mouse from Riken testis EST evidence
•350 aa-human "hypothetical" with 65% identity to mouse
•no other significant BLASTP hits to any known proteins

2° •both mouse and human predicted to be membrane proteins with 2 trans membrane domains



NORSS-PEPTIDE ANTIBODY-PEPTIDE IDED
SMVGPEDAGNYRC

TRANSMEMBRANE DOMAIN~22 aa

PREDICTED DISULFIDE BRIDGES

* POTENTIAL PHOSPHORYLATION SITES
• PKA, PKC, CKII

MULTIPLE POST-TRANSLATIONAL MODS PREDICTED

MOUSE SPERM PLASMA MEMBRANE

FIG. 6

PAIRWISE ALIGNMENT OF MOUSE AND HUMAN BAND 5 PROTEINS

46% IDENTICAL

65% SIMILAR

```
MOUSE   1   MGPHFTLLLAALANCLCPGRPCTKCDQFVTDALKTFENTYLNDHLPHDIH
HUMAN   1   MGPHFTLLCAALAGCLLPAEGCVICDPSVVLALKSLEKDYLPGHLDAKHH

MOUSE  51   KNVMRMVNHEVSSFGVVTSAEDSYLGAVDENTLEQATWSFLKDLKRTTDS
HUMAN  51   KAMMERVENAVKDFQELSLNEDAYMGVVDEATLQKGSWSLLKDLKRITDS

MOUSE 101   DLKGELFIKELLWMLRHQKDIFNNLARQFQKEVLCPNKCGVMSQTLIWCL
HUMAN 101   DVKGDLFVKELFWMLHLQKETFATYVARFQKEAYCPNKCGVMLQTLIWCK

MOUSE 151   KCEKQLHICRKSLDCGERHIEVHRSEDLVLDCLLSWHRASKGLTDYSFYR
HUMAN 151   NCKKEVHACRKSYDCGERNVEVPQMEDMILDCELNWHQASEGLTDYSFYR

MOUSE 201   VWENSSETLLAKGKEPYLTKSMVGPEDAGNYRCVLDTINQGHATVTRYDV
HUMAN 201   VWGNNTETLVSKGKEATLTKPMVGPEDAGSYRCELGSVNSSPATIINFHV

MOUSE 251   TVLPPKHSEENQPPNIITQEEHETPVHVTPQTPPGQEPESELYPELHPEL
HUMAN 251   TVLPKMIKEEKPSPN-I----------V---T-PG---EATT----ESSI

MOUSE 301   YPELIPTVAQNPEKKMKTRLLILLTLGFVVLVASIIISVLHFRKVSAKLK
HUMAN 278   -S-LQP---LQPEKMLASRLLGLLICGSLALITGLTFAIFRRRKVIDFLK

MOUSE 350   -N--A-SDEVKPTASGSKSDQSLSQQMGLKKASQADFNSDYSGDKSEATE
HUMAN 324   SSLFGLGSGVAEQTQVPKEKATDSRQQ--------------------

MOUSE 397   N
HUMAN 350   -
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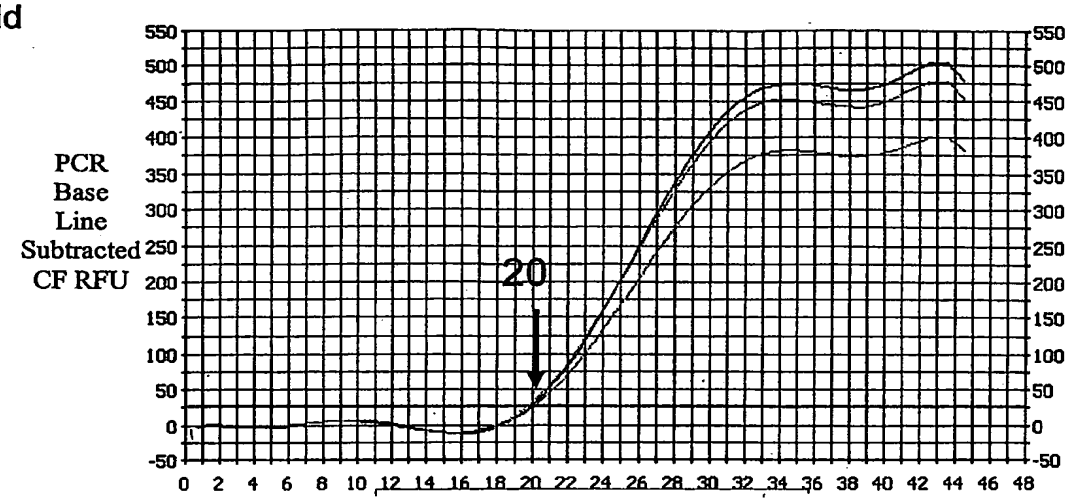
AT THE cDNA LEVEL THE TWO ARE 75% IDENTICAL BY BLAST ALIGNMENT

FIG. 7A

REAL-TIME PCR OF MOUSE TESTIS cDNA

Threshold Cycle

The lower this cycle the greater the initial amount of cDNA

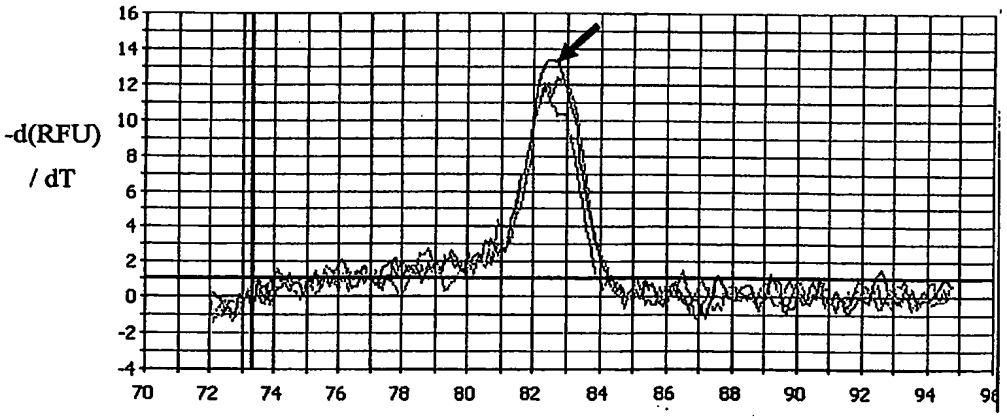


PCR cycle where product fluorescence crosses threshold

FIG. 7B

Melt Curve

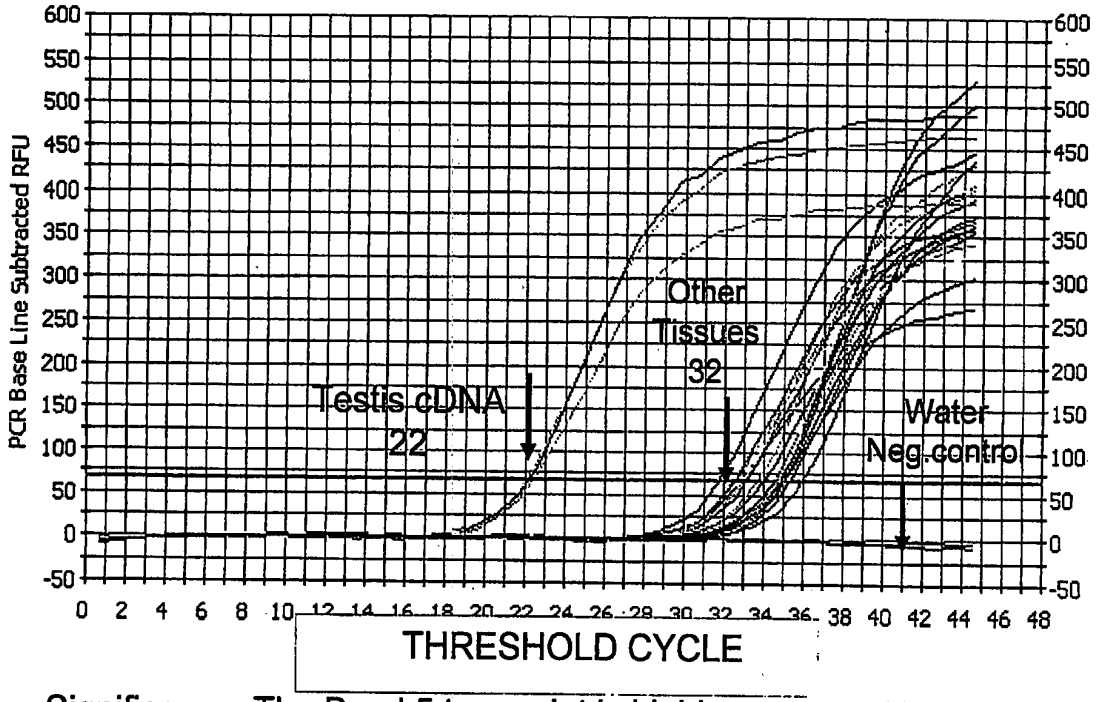
Single peak implies single product



Temperature where product melts

FIG. 8

REAL-TIME PCR MOUSE MULTIPLE TISSUE cDNA PANEL SCREEN



Significance: The Band 5 transcript is highly expressed in testis

In Other Tissues: Heart, Brain, Liver, Lung, Kidney, Pancreas, Skeletal Muscle
The threshold cycle is 10 to 14 cycles later suggesting a much lower level of transcript expression.

FIG. 9

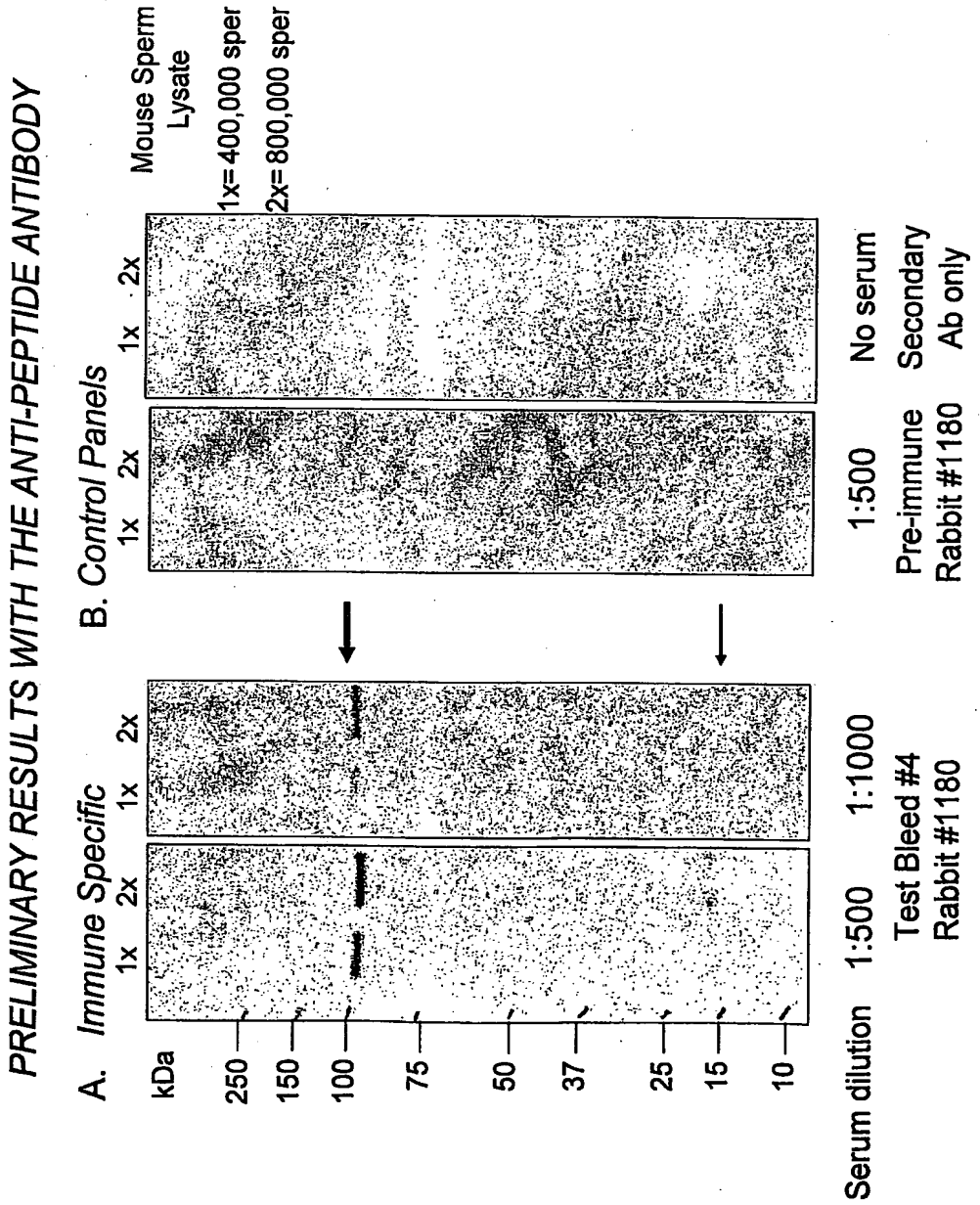
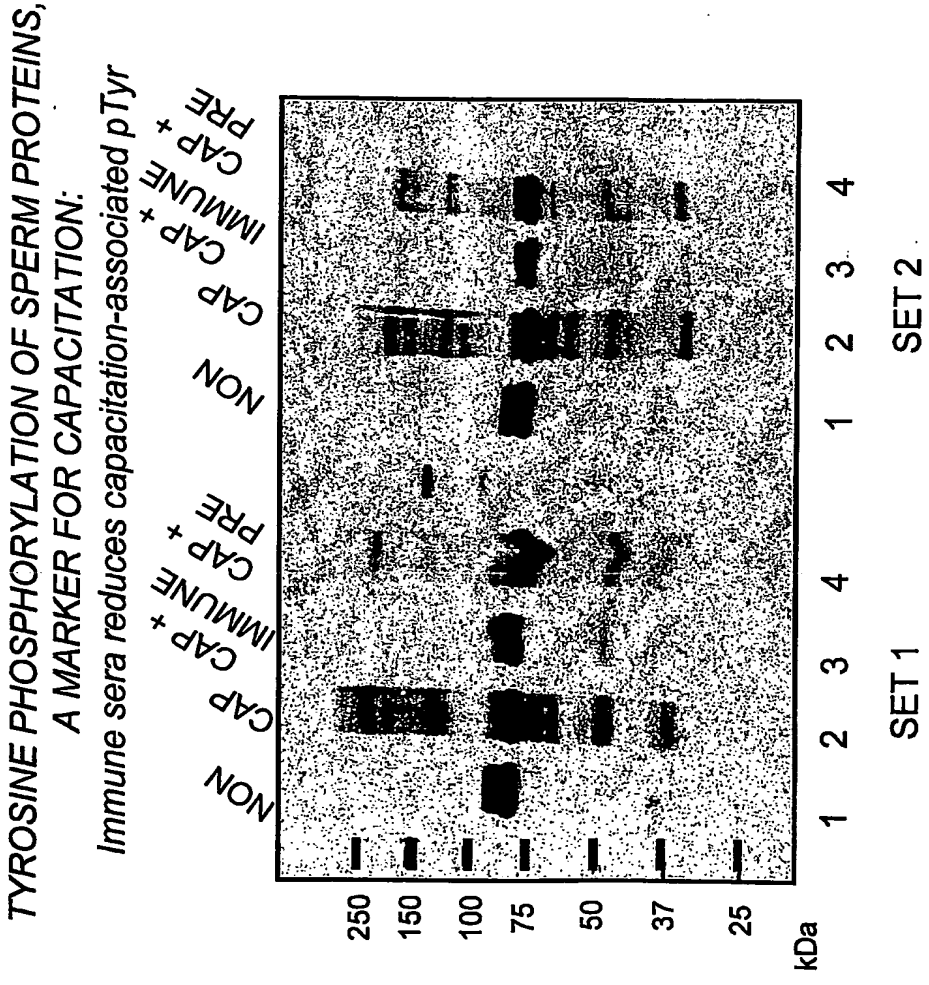

PRELIMINARY RESULTS WITH THE ANTI-PEPTIDE ANTIBODY
A. Immune Specific
B. Control Panels
Mouse Sperm Lysate
1x= 400,000 sper
2x= 800,000 sper
1x 2x
kDa
250
150
100
75
50
37
25
15
10
Serum dilution 1:500 1:1000
Test Bleed #4
Rabbit #1180
1:500
Pre-immune
Rabbit #1180
No serum
Secondary Ab only

FIG. 10


TYROSINE PHOSPHORYLATION OF SPERM PROTEINS,
A MARKER FOR CAPACITATION:
Immune sera reduces capacitation-associated pTyr
NON
CAP
CAP + IMMUNE
CAP + PRE
NON
CAP
CAP + IMMUNE
CAP + PRE
250
150
100
75
50
37
25
kDa
1 2 3 4 1 2 3 4
SET 1
SET 2